



Supplementary Figure 1. Original blots from figures. Dashed box indicates cropped images shown in the corresponding figures.

HFD increases ISC proliferation

The fact that HFD boosted the numbers of ISCs and crypt depth in the small intestine suggested that it augments the proliferation of ISCs and their more differentiated progeny (i.e. progenitors), which reside in intestinal crypts. To address this possibility, we assessed incorporation of 5-bromodeoxyuridine (BrdU) into ISCs and progenitors. After a 4-hour pulse of BrdU, crypts of HFD mice had more BrdU⁺ stem cells compared to controls (Fig. 1b, Extended Data 1h, i; and for the colon, Extended Data 3f). Furthermore, HFD augmented the number of BrdU⁺ cells in the larger pool of progenitors (Fig. 1b, Extended Data 1i), indicating that output and migration from this compartment into villi may also increase. Indeed, HFD mice 24 hour after a single dose of BrdU had more absolute numbers of BrdU-labeled cells in the villi (Extended Data 1j, k) and a greater percentage of villus enterocytes that were BrdU⁺ (Extended Data 1j, k). These findings demonstrate that the increase in stem and progenitor cell proliferation induced by HFD translates into enhanced output and migration of crypt cells into the villus.

A HFD and PPAR-d signaling increase β -catenin activity

Single cell analyses of β -catenin target gene expression revealed heterogeneity in control and HFD-derived ISCs and progenitors (Extended Data 8a). t-Distributed stochastic neighbor embedding (t-SNE) analysis of single cell gene expression data of all β -catenin target genes demonstrated clustering of HFD-derived ISCs and progenitors (Extended Data 8d) from their control-fed counterparts, consistent with the notion that HFD induces β -catenin target gene expression in intestinal stem and progenitor cells. Furthermore, we found that while HFD-induced β -catenin signature genes (HFD signature; Extended Data 8c) were heterogeneously

expressed in control ISCs and progenitors, they formed a distinct cluster upon HFD as shown in the t-SNE analysis (Extended Data 8f) and by qRT-PCR in the colon (Extended Data 3i).

Analysis of leptin receptor deficient *db/db* obese mice

In contrast to controls, small intestinal mass, length, and villi length increased, while crypt depth, ISC and Paneth cell numbers decreased in *db/db* mice (Extended Data 9a-h). Although organoid-forming capacity of *db/db* crypts trended higher relative to controls (Extended Data 9i), there was no increase in HFD-induced PPAR-d or β -catenin target gene expression in *db/db* intestinal progenitors (Extended Data 9j). Overall, these findings are mostly opposite to what we observed in HFD mice and highlight that, even in obesity, diet impacts intestinal stem and progenitor biology.

Enforced PPAR-d signaling permits non-*Lgr5*⁺ cells to initiate intestinal adenomas

The finding that *Apc*-loss after enforced PPAR-d signaling does not have additive effects on tumor initiation indicates that in *Apc-wild type* stem cells enforced PPAR-d signaling drives many of its effects (e.g. reduced Paneth cell dependence in the assay) through beta-catenin signaling (Fig. 5g, h). In the *Apc*-null state, it is interesting to note that enforced PPAR-d signaling differentially affects the adenoma-initiating capacity of stem and progenitor cells upon *Apc* loss (Fig. 5g, h). One possible explanation for this divergence may be that ISCs, in contrast to progenitors, are in a cellular state that is facilitative for β -catenin signaling. Progenitors, however, are in a more differentiated cellular state that is less responsive to β -

catenin signaling, and perhaps enforced PPAR-d signaling in a subset of progenitors augments this responsiveness—thus enabling them to initiate tumors upon *Apc*-loss.

Supplementary Table 1 | Primer sequences for qRT-PCR

Gene	Forward Primer	Gene	Reverse Primer
Pparg_F	CCACCAACTCGGAATCAGCT	Pparg_R	TTTGTGGATCCGGCAGTTAAGA
Ppara_F	AGAGCCCCATCTGCTCTC	Ppara_R	ACTGGTACTGCAAACCAAA
Ppard_F	TCCATCGTCAACAAAGACGGG	Ppard_R	ACTTGGGCTCAATGATGTCAC
Mgll_F	CGGACTTCCAAGTTTGTCAGA	Mgll_R	GCAGCCACTAGGATGGAGATG
Slc22a3_F	CGTTTCTGCTTTGGCTG	Slc22a3_R	TGCAACTGTGAACTGCCAAG
Fabp1_F	GGGAAGAAAATCAAACACCAC	Fabp1_R	AGTTGTCACCATTTATTGTCACC
Pdk4_F	AGGGAGGTCGAGCTGTTCTC	Pdk4_R	GGAGTGTTCACTAACGGGTCA
Hmgcs2_F	ATACACCAACGCCGTTATGG	Hmgcs2_R	CAATGTCACCAACAGACCACAG
Cpt1a_F	CCATGAAGCCCTAAACAGATC	Cpt1a_R	ATCACACCCACCACACGATA
Actb_F	GGCTGTATTCCCTCCATCG	Actb_R	CCAGTTGGTAACGCCATGT
mtCytB_F	CATTTATTATCGCGGCCCTA	mtCytB_R	TGTTGGGTTGATCCTG
bglobin_F	GAAGGCATTCTAGGGACAG	bglobin_R	GGAGCAGCGATTCTGAGTAGA
II6_F	TAGTCCTCCCTACCCCAATTCC	II6_R	TTGGTCCTTAGCCACTCCTTC
II1b_F	GCAACTGTTCCCTGAACTCAACT	II1b_R	ATCTTTGGGTCCGTCAACT
Tnfa_F	CCCTCACACTCAGATCATCTCT	Tnfa_R	GCTACGACGTGGGTACAG
Ifng_F	ATGAACGCTACACACTGCATC	Ifng_R	CCATCCTTTGCCAGTCTC
Apc-Int13FloxF	GAGAAACCCCTGTCGAAAAAA	Apc-Int13FloxF_R	AGTGCTGTTCTATGAGTCAC
Apc-Int13F2_del_F	GAGAAACCCCTGTCGAAAAAA	Apc-Int14R4_del_R	TTGGCAGACTGTGTATAAGC
qCD24a_F	TTCTGGCACTGCTCCATTAC	qCD24a_R	GCGTTACTTGGATTGGGAA
qChga_Fwd	CCAAGGTGATGAAGTGGCTC	qChga_Rev	GGTGTCCGAGGATAGAGAGGA
Ppard_del_F	TGTGCAGACCTCTCCAGAA	Ppard_del_R	CCGACATTCCATGTTGAGGC
Lyz_F	GAGACCGAAGCACCAGACTATG	Lyz_R	CGGTTTGACATTGTTGCGC
Muc2_F	ATGCCAACCTCCTAAAGAC	Muc2_R	GTAGTTCCGTTGAAACAGTGA
human_Cpt1a_F	TCCAGTTGGCTTATCGTGGTG	human_Cpt1a_R	TCCAGAGTCCGATTGATTTTG
human_Pdk4_F	GGAGCATTCTCGCGCTACA	human_Pdk4_R	ACAGGCAATTCTGCGCAA
human_Hmgcs2_F	GACTCCAGTGAAGCGCATTCT	human_Hmgcs2_F	CTGGGAAGTAGACCTCCAGG
human_Fabp1_F	GTGTCGGAATCGTCAGAAT	human_Fabp1_R	GACTTCTCCCTGTCATTGTC

Supplementary Table 2 | Primer sequences for single cell analysis

SEQUENCE NAME	Forward Primer	SEQUENCE NAME	Reverse Primer
ABCB1F	CAGCAGTCAGTGTGCTTACAA	ABCB1R	ATGGCTCTTTATCGGCCTCA
ADAM10F	ATGGTGTGCCGACAGTGT	ADAM10R	GTTTGGCACGCTGGT
ALEX1F	CTGGTGCCTGCTACTGTG	ALEX1R	CCCCTACCCCAACATTAGT
ASCL2F	AAGCACACCTTGACTGGTACG	ASCL2R	AAGTGGACGTTGCACCTTCA
AXIN2F	TGACTCTCCCTCAGATCCC	AXIN2R	TGCCCACACTAGGCTGACA
BAMBIF	GATGCCACTCCAGCTACTTC	BAMBIR	GCAGGCACTAACGCTAGACTT
BCL2L2F	GCGGAGTTCACAGCTTAC	BCL2L2R	AAAAGGCCCTACAGTTACCA
BCL2L1F	GACAAGGAGATGCAGGTATTG	BCL2L1R	TCCCCTAGAGATCCACAAAGT
BIRC5F	GAGGCTGGCTCATCCACTG	BIRC5R	CTTTTGCTTGTGTTGGCTCC
BMI1F	ATCCCCACTTAATGTGTCCT	BMI1R	CTTGCTGGCTCCAAGTAACG
BMP4F	TTCTGGTAACGAATGCTGA	BMP4R	CCTGAATCTCGCGACTTTT
CCND1F	GCGTACCCCTGACACCAATCTC	CCND1R	CTCCTCTCGCAGTCTGCTC
CD44F	CACCATTGCCCTCAACTGTG	CD44R	TTGTGGCTCTGAGTCTGA
CDKN2AF	CGCAGGTTCTGGTCACTGT	CDKN2AR	TGTTCACGAAAGCCAGAGCG
CDX1F	GGACGCCCTACGAATGGATG	CDX1R	GTACCGGCTGTAGTGAAC

CLDN1F	GGGGACAACATCGTGACCG	CLDN1R	AGGAGTCGAAGACTTGCAC
COX2F	TGAGCAACTATTCCAAACCAGC	COX2R	GCACGTAGTCTCGATCACTATC
DKK1F	CTCATCAATTCCAACGCCATCA	DKK1R	GCCCTCATAGAGAACCTCCG
DKK4F	GTACTGGTACCTGCTTGG	DKK4R	CCGTTCATCGTAAACGCTAAG
DNMT1F	AAGAATGGTGTGCTACCGAC	DNMT1R	CATCCAGGTTGCTCCCTTG
EDN1F	GCACCGGAGCTGAGAATGG	EDN1R	GTGGCAGAAGTAGACACACTC
EFNB1F	TGTGGCTATGGTCGTGCTG	EFNB1R	CCAAGCCCTCCACTTAGG
ENC1F	CTGTTCTATAAGTCCTCTACGC	ENC1R	CACCACTGAACATGGCTCG
EPHB2F	GCGGCTACGACGAGAACAT	EPHB2R	GGCTAAGTCAAATCAGCCTCA
EPHB3F	CATGGACAGGAAATGGGTGAC	EPHB3R	GCGGATAGGATTCATGGCTTC
FGF18F	CCTGCACTTGCCTGTGTTAC	FGF18R	TGCTTCCGACTCACATCATCT
FGFBP1F	CTAAATCTCTGACGCATGGCA	FGFBP1R	AACTCCTGATCGGTTGTGTG
FGFBP3F	GGTCGCTCGTGAGTCCAG	FGFBP3R	AGCAGCCGCTCCAGTAGT
FRA1F	ATGTACCGAGACTACGGGAA	FRA1R	CTGCTGCTGTCGATGCTTG
FSCN1F	GAUTGCGAAGGTGCGTAC	FSCN1R	CTGATCGGTCTTCTACCTGA
GASTF	CGCTCCCAGCTACAGGATG	GASTR	GGTCTGCTATGAAGTGTGAGG
HATH1F	GAGTGGGCTGAGGTAAAAGAGT	HATH1R	GGTCGGTCTATCCAGGAG
NEDD9F	ATGTGGCGAGGAATCTTATGG	NEDD9R	TTCCCTGGGACAATGCCCTG
HES1F	CCAGCCAGTGTCAACACGA	HES1R	AATGCCGGGAGCTATCTTCT
ID2F	ATGAAAGCCTTCAGTCCGGTG	ID2R	AGCAGACTCATGGTCGT
TCF4F	CGAAAAGTCCCTCCGGGTTG	TCF4R	CGTAGCCGGGCTGATTCAT
JAG1F	CCTCGGGTCAGTTGAGCTG	JAG1R	CCTTGAGGCACACTTGAAGTA
JUNF	CCTTCTACGACGATGCCCTC	JUNR	GGTTCAAGGTATGCTCTGTT
L1CAMF	AAAGGTGCAAGGGTGACATT	L1CAMR	TCCCCACGTTCTGTAGGT
LAMC2F	CAGACACGGGAGATTGCTACT	LAMC2R	CCACGTTCCCAAAGGGAT
LEF1F	TGTTTATCCCACACGGTGG	LEF1R	CATGGAAGTGTGCCTGACAG
LGR5F	CCTACTCGAAGACTTACCCAGT	LGR5R	GCATTGGGTGAATGATAGCA
MENAF	GCCCCAGAGCAAGGTTACTG	MENAR	GCCCACAGAAAATACATCGCAA
METF	GTGAACATGAAGTATCAGCTCC	METR	TGTAGTTGTGGCTCCGAGAT
MMP14F	CAGTATGGCTACCTACCTCCAG	MMP14R	GCCTTGCTGTCACTGTAAA
MMP7F	CTGCCACTGTCCCAGGAAG	MMP7R	GGGAGAGTTTCCAGTCATGG
MYBF	AGACCCCGACACAGCATCTA	MYBR	CAGCAGCCATCGTAGTCAT
MYCF	ATGCCCTCAACGTGAACTTC	MYCR	CGCAACATAGGATGGAGAGCA
MYCBPF	GCTGGACACGCTGACGAAA	MYCBPR	TCTAGGCGAACAGCTCTATT
NOS2F	GTTCTCAGCCCAACAATACAAGA	NOS2R	GTGGACGGTCGATGTCAC
NOTCH2F	ATGTGGACGAGTGTCTGTTGC	NOTCH2R	GGAAGCATAGGCACAGTCATC
NRCAMF	AAAGGGAAACCTCCCCAAG	NRCAMR	TGTTGATGACAAGGTTCTGA
PLAUF	GCGCCTGGTGGTGGAAAAAC	PLAUR	TTGTAGGACACGCATACACCT
PLAURF	CAGAGCTTCCACCGAATGG	PLAURR	GTCCCCGGCAGTTGATGAG
PPARDF	TCCATCGTCAACAAAGACGGG	PPARDR	ACTTGGGCTCAATGATGTCAC
S100A4F	TCCACAAATACTCAGGCAAAGAG	S100A4R	GCAGCTCCCTGGTCAGTAG
SGK1F	CTGCTCGAAGCACCCTTACC	SGK1R	TCCTGAGGATGGACATTTCA
SMC3F	CGAAGTTACCGAGACCAAACA	SMC3R	TCACTGAGAACAAACTGGATTGC

SOX9F	AGTACCCGCATCTGCACAAC	SOX9R	ACGAAGGGTCTTCTCGCT
SP5F	TGGGTTCACCCCTCCAGACTTT	SP5R	CCGGCGAGAACTCGTAAGG
SRSF3F	GCGCAGATCCCCAAGAAGG	SRSF3R	ATCGGCTACGAGACCTAGAGA
SUZ12F	AACTCGAAATCTTATCGCACCAA	SUZ12R	TGCAAATGTGCAGACAAGCTAT
TCF1F	AGGAGTGTAAATAGGGCGGAGT	TCF1R	GAGGTCCGTTATAGGTGTCCA
TIAM1F	GAAGCACACTTCACGCTCC	TIAM1R	CTCCAGGCCATTTCAGCCA
TNCF	ACGGCTACCACAGAACGCTG	TNCR	ATGGCTGGTTGCTATGGCA
VEGFF	GCCAGACAGGGTGCACATAC	VEGFR	GGAGTGGATGGATGATGTCAG
YAP1F	TACTGATGCAGGTACTGCGG	YAP1R	TCAGGGATCTCAAAGGAGGAC
CCND3F	CGAGCCTCCTACTTCCAGTG	CCND3R	GGACAGGTAGCGATCCAGGT
FZD1F	CAGCAGTACAACGGCGAAC	FZD1R	GTCCTCCTGATTGTTGGC
FZ7F	GCCACACGAACCAAGAGGAC	FZ7R	CGGGTGCACATAGAGCATAA
BTRCPF	AAGACTGTAATAATGGCGAACCC	BTRCPR	TCTCTGGTTATGCAAAGCCTG
BCATENINF	ATGGAGCCGGACAGAAAAGC	BCATENINR	CTTGCCACTCAGGGAGGA
NEMOF	AAGCACCCCTGGAAGAAC	NEMOR	CCTGCTCTGAAGGCAGATGTA
NAKED1F	AGGAAAGGCATCGAGGAGTG	NAKED1R	TCGCTCAGTCTCTCCATTCTC
RNF43F	TCCGAAAGATCAGCAGAACAGA	RNF43R	GGACTGCATTAGCTCCCTTC
LRP6F	TTGTTGCTTATGCAAACAGACG	LRP6R	GTTCGTTAATGGCTTCTCGC
LRP5F	AAGGGTGCTGTACTGGAC	LRP5R	AGAAGAGAACCTTACGGGACG
OLFM4F	CAGCCACTTCCAATTCACTG	OLFM4R	GCTGGACATACTCCTCACCTTA
PREX1F	TTAACCAAGGTCGATTCATCCA	PREX1R	CGGACCGTGCATTCCCTTT
RAC1F	GAGACGGAGCTGGTAAAA	RAC1R	ATAGGCCAGATTCACTGGTT
Clnd15F	ATGTCGGTAGCTGTGGAGAC	Clnd15R	GGACGGAAAGTCCCAGCAG
Edn3F	CCCTGGTGAGAGGATTGTGTC	Edn3R	CCTTGTCTTGTAAAGTGAAGCAC
EFNB2F	ATTATTGCCCAAAGTGGACTC	EFNB2R	GCAGCGGGTATTCTCCCTTC
EPHB4F	TATGCCACGATACGCTTCACC	EPHB4R	AGCTTCGCTCTCGTAATAGAAGA
FGFR4F	GCTCGGAGGTAGAGGTCTTGT	FGFR4R	CCACGCTGACTGGTAGGAA
NEDD8F	GGAGCGAATCAAGGAGCGT	NEDD8R	ACGGAACCACCTAGAATCTTGT
TCF7F	AGCTTCTCCACTCTACGAACA	TCF7R	AATCCAGAGAGATCGGGGTC
JAG2F	CAATGACACCACTCCAGATGAG	JAG2R	GGCCAAAGAAGTCGTTGCG
SMC2F	GGCTGGATTACCAAAGCCTC	SMC2R	CACCAATAACCACCTGTCTTGT
Sox9F	GAGCGGATCTGAAGAGGGA	Sox9R	GCTTGACGTGTGGCTTGTTC
NcadherinF	ATGTGCCGGATAGCGGGAGC	NcadherinR	TACACCGTGCCGTCCCTCGTC
EcadherinF	CACCTGGAGAGAGGCCATGT	EcadherinR	TGGGAAACATGAGCAGCTCT
ACTINBF	GGCTGTATTCCCTCCATCG	ACTINBR	CCAGTTGTAACAATGCCATGT
GAPDHF	AGGTCGGTGTGAACGGATTG	GAPDHR	TGTAGACCATGTAGTTGAGGTCA